Subtribe Pterostylidinae: A multidisciplinary approach to investigating and resolving taxonomic confusion

By

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~Declarations~

Statement of originality

I declare that this thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

Jasmine Karla Janes
2nd February 2010

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~Abstract~

The distribution, niche-partitioning, taxonomy and population-level genetic variation of Tasmanian representatives of the subtribe Pterostylidinae were investigated. General ecological data were collected over a two year period to assess changes in the known distribution of *Pterostylis* species within Tasmania and identify previously unknown populations. Ecological characteristics relating to habitat were used to perform a series of canonical correspondence analyses (CCA) to effectively delimit each species' ecological tolerances and niche hyperspace. A series of Bayesian and maximum parsimony phylogenetic reconstructions were conducted using DNA sequence data from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA to elucidate generic and infrageneric relationships within the Pterostylidinae. Following from these results, the genetic variation within the Tasmanian members of the "longifolia" species complex was investigated using amplified fragment length polymorphism (AFLP) and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP).

Ecological survey data indicated that the distribution and abundance of *Pterostylis* species within Tasmania had changed significantly within the past 10 years, with different species apparently more or less common than previously thought. In addition to the number of populations located, the number of individuals within each population provided insight into the overall level of conservation status applicable to particular species and several recommendations for the management of these species were made. Niche partitioning based on habitat characteristics revealed differences in the ecological tolerances of *Pterostylis* species and effectively delimited many species along ecological gradients. Significant overlap in the fundamental niches of several morphologically similar species was identified, which suggested that some taxa within Pterostylidinae complexes had been incorrectly assigned to the level of species. This hypothesis was later confirmed through a phylogenetic and population genetic study.

Phylogenetic work using ITS sequence data confirmed that Pterostylidinae is embedded within the tribe Cranichideae. Furthermore, the reconstructions strongly supported a monotypic subtribe (Pterostylidinae) comprising the single genus *Pterostylis* R.Br *sensu lato*. The analysis found two strongly supported clades that correlated with the morphology of the lateral sepal position within the subtribe and, are herein, delimited as subgenera of *Pterostylis*. Several closely related species from

within species complexes had identical ITS sequences; together with the CCA data the results indicate the presence of over-splitting of the Pterostylidinae.

PCR-RFLP analysis of the chloroplast genome in the “longifolia” species complex revealed extremely low variation among species and no marker was found that could distinguish between the Tasmanian representatives of this complex. Further work at the population level using AFLP markers indicated high levels of polymorphism within the complex but, again, could not delimit the four “longifolia” species. Instead, population structure analysis revealed geographic separation of “longifolia” populations irrespective of species. The information from these investigations is essential for taxonomic resolution within the subtribe Pterostylidinae and has clear implications for *Pterostylis* conservation and management.

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Several dedicated people assisted me in the field and laboratory. **James Marthick** (aka The Extractinator) spent many hours grinding samples for DNA extraction and became a proficient orchid spotter – he also suggested several alternative titles for this thesis! **Louise Ryall** saw more of Tasmania in one year than many Tasmanians see in their lives and I am pleased to say that I introduced her to her first leech. **Hans and Annie Wapstra**, and their son **Mark** provided a wealth of information!! The Wapstras freely shared their knowledge of orchid habitat, locations and enthusiasts; without this information I might still be crawling around in the bush somewhere in the northeast of Tasmania. **Peter Norris**, from the Department of Defence Fort Direction property, enthusiastically organised for me to collect specimens and happily assisted. **Allegra Biggs-Dale** allowed me to tour her Bruny Island property for sample collection and was most accommodating.

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~Preface~

Very little orchid research has been conducted within Tasmania and it is only within the last 10 years that orchids have become the focus of many studies within Australia. As a result of the paucity of orchid related studies, particularly within Tasmania, there were many more questions being asked than could possibly be incorporated into this thesis. The greenhoods (subtribe Pterostylidinae) were chosen for several reasons. Greenhoods are a taxonomically difficult group with a high degree of endemism and presence in Tasmania. The group also comprises a large portion of threatened orchid species within Tasmania. But perhaps most important from a thesis perspective, greenhoods, as a group, display a relatively staggered flowering pattern. This pattern of flowering made it possible to plan seasonal field trips in which particular species were targeted for collection and enabled the collection of the majority of *Pterostylis* species occurring in Tasmania.

This thesis has been structured in such a way that each chapter relates to a specific topic. "Chapter One: An introduction to the Orchidaceae" introduces orchidology and its history – what makes orchidology so fascinating and so complex. The subtribe Pterostylidinae and the Tasmanian greenhood species are introduced in Chapter One. A portion of this chapter, namely the history of orchidology, was published in *The Orchadian* in 2006.

"Chapter Two: Occurrence and conservation status of Tasmanian *Pterostylis*" provides a comprehensive account of the current greenhood distribution and abundance within Tasmania. This chapter also provides several recommendations for the conservation management of Tasmanian greenhood populations and was published in the *Tasmanian Naturalist* in 2008.

"Chapter Three: Habitat requirements of *Pterostylis*" details the ecological requirements of several *Pterostylis* species within Tasmania. Extensive canonical correspondence analyses were performed to identify the fundamental niches of several species according to the most informative ecological gradients. The results from Chapter Three are currently being written up as a paper for submission to an appropriate peer-reviewed journal.

"Chapter Four: A re-evaluation of Pterostylidinae genera" reconstructs phylogenetic trees of representative *Pterostylis* species from ITS sequence data. From this chapter,

a new, informative classification is proposed that accurately accounts for the morphological and evolutionary variation within the subtribe. The results from Chapter Four have resulted in two papers: one that is identical in structure and content to Chapter Four that is currently under review for the peer-reviewed journal *Molecular Phylogenetics and Evolution*, whilst the second is a formal taxonomic classification that is currently being prepared for submission to *Taxon*.

"Chapter Five: Population genetic structure in the Tasmanian *Pterostylis longifolia* complex" investigates the level of genetic variation and structure within the four Tasmanian representatives. This chapter made use of the DNA profiling techniques AFLP (genomic DNA) and RFLP (chloroplast DNA) to discriminate between very closely related taxa. Results from Chapter Four will be formatted in accordance with submission requirements of a suitable peer-reviewed journal at a later date.

"Chapter Six: Towards an understanding of the Tasmanian Pterostylidinae" provides a general discussion and review of the results presented in the preceding chapters. A large section of this chapter is dedicated to recommending areas of future research in order to gain a more thorough understanding of the evolutionary processes at work within the Pterostylidinae.

"References" provides a full reference list of the citations from each chapter. This chapter is followed by Appendix I which details the genetic distance pairwise matrix between populations referred to in Chapter Five.

~Publications arising from this thesis~

- Janes JK (2006). A short review on the history of orchid taxonomy. *The Orchadian*. **15**:6, 252-255

- Janes JK, Vaillancourt RE, Steane DA (2008). The occurrence and conservation status of Tasmanian *Pterostylis* (Orchidaceae). *Tasmanian Naturalist*. **130**, 86-99.

- Janes JK, Steane DA, Vaillancourt RE, Duretto MF (2009). A molecular phylogeny of the subtribe Pterostylidinae: resolving the taxonomic confusion (under review with *Molecular Phylogenetics and Evolution*; submitted 24 July 2009).

- Janes JK, Duretto MF (2009). A new classification for subtribe Pterostylidinae (orchidaceae): the reaffirmation of *Pterostylis* R.Br. *sensu lato* (accepted 21 January 2010 in *Australian Systematic Botany*).

- Janes JK, Steane DA, Vaillancourt RE (2010). Ecological requirements and niche partitioning of Pterostylidinae (Orchidaceae) species (submitted 29 January 2010 to *Austral Ecology*).

~Index to acronyms~

AFLP	Amplified Fragment Length Polymorphism
AGL	alpine grassland
AMOVA	Analysis of Molecular Variance
BL	branch length
BP	bootstrap support
C	Central Plateau area in Tasmania
CAL	Callitris forest
CCA	canonical correspondence analysis
CH	coastal heath
CI	consistency index
cpDNA	chloroplast genome
CR	critically endangered under EPBCA
DCA	detrended correspondence analysis
DNA	deoxyribonucleic acid
DPIW	Department of Primary Industries and Water, Tasmania
DS	dry sclerophyll forest
DS-CH	dry sclerophyll forest with coastal heath aspects
DS-S	dry sclerophyll forest with sedgeland understorey
e	endangered under TTSPA
EN	endangered under EPBCA
EPBCA	Environmental Protection and Biodiversity Conservation Act 1999
f	inbreeding coefficient, analogous to F_{is} (Holsinger and Lewis 2005)
FI	Flinders Island
F_{ST}	level of genetic differentiation among populations
GL	grassland
GPS	Global Positioning System
HO	Hobart Herbarium, Tasmania
ITS	internal transcribed spacer of nuclear ribosomal DNA
IUCN	International Union for the Conservation of Nature
K	number of clusters in genetic population (Chen *et al.* 2007)
KI	King Island
MP	maximum parsimony
nDNA	nuclear genome
NE	northeast

NJ	Neighbour Joining Tree
NP	national park
NVA	Natural Values Atlas, DPIW database
NW	northwest
PCoA	Principle Coordinates Analysis
PCR	polymerase chain reaction
PP	posterior probability
r	rare under TTSPA
RI	retention index
RFLP	Restriction Fragment Length Polymorphism
SE	southeast
TTS	tea tree scrub
TTSPA	Tasmanian Threatened Species Protection Act 1995
v	vulnerable under TTSPA
VU	vulnerable under EPBCA
WL-S	wetland with sedgeland
WS	wet sclerophyll forest
x	extinct under TTSPA
Θ^{II}	level of genetic differentiation among populations, analogous to F_{ST} (Holsinger and Lewis 2005)

~Contents~

Some are incredibly beautiful - - almost magical. [A *Cattleya* orchid] … only blooms when it is motivated. I've had it for 10 years now. Sometimes I wonder why I keep it. I've forgotten what it looks like so I'll just wait and see when it does decide to bloom.

- Elenor Mis on orchids